# Deuteration of solid-state NMR samples for structural measurements with weakly-binding ligands and membrane proteins

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## Introduction

Solid-state NMR is unique for its ability to observe directly the binding of ligands to membrane proteins in their native membranes or purified and reconstituted in detergent micelles or lipids. This is made possible by using an isotope-labelled ligand (usually containing $^{13}C$, $^{15}N$, $^{19}F$ or $^{31}P$) with the cross-polarisation magic-angle spinning (CP-MAS) experiment. Dipolar recoupling experiments can then be used to measure ligand-protein interactions with isotope labels incorporated at specific positions in the protein or precise intra-molecular distances and torsion angles within the ligand to elucidate its protein-bound structure. These experiments are more challenging when there is a significant non-specific component to the ligand signal. For a system that has a weakly-binding (high µM to mM affinity) ligand, the principal potential source of a non-specific component comes from excess unbound ligand frozen in solution, as structural measurements are usually performed with the sample at a temperature of less than -10 ºC. We have investigated deuteration as a novel way to suppress or eliminate this non-specific signal as demonstrated with the *E. coli* sugar transport protein GalP and its substrate D-glucose.

## Results

The principle of our approach for using deuteration to eliminate the non-specific components of ligand signals from CP-MAS solid-state NMR spectra is outlined in Figure 1 for a membrane protein sample with a $^{13}C$-labelled ligand. **A.** Shows the pulse sequence for a $^{1}H$-$^{13}C$ cross-polarisation experiment, which transfers magnetisation from $^{1}H$ nuclei to nearby $^{13}C$ nuclei and then the magnetisation on $^{13}C$ nuclei is detected to give signals in the $^{13}C$ spectrum. **B.** Shows the potential sources of $^{1}H$-$^{13}C$ CP interactions that can contribute to the observed ligand signal when a $^{13}C$-labelled ligand is used with a membrane sample in $H_2O$. The main sources of $^{1}H$ magnetisation are: (i) $^{1}H$ in the ligand backbone; (ii) $^{1}H$ in ligand hydroxyl groups (or other exchangeable groups); (iii) $^{1}H$ in water molecules that are frozen/immobilised (non-specific); (iv) protein non-exchangeable $^{1}H$ (specific binding); (v) protein exchangeable $^{1}H$ (specific binding); (vi) membrane non-exchangeable $^{1}H$ (non-specific) and (vii) membrane exchangeable $^{1}H$ (non-specific). **C.** Shows how the majority of

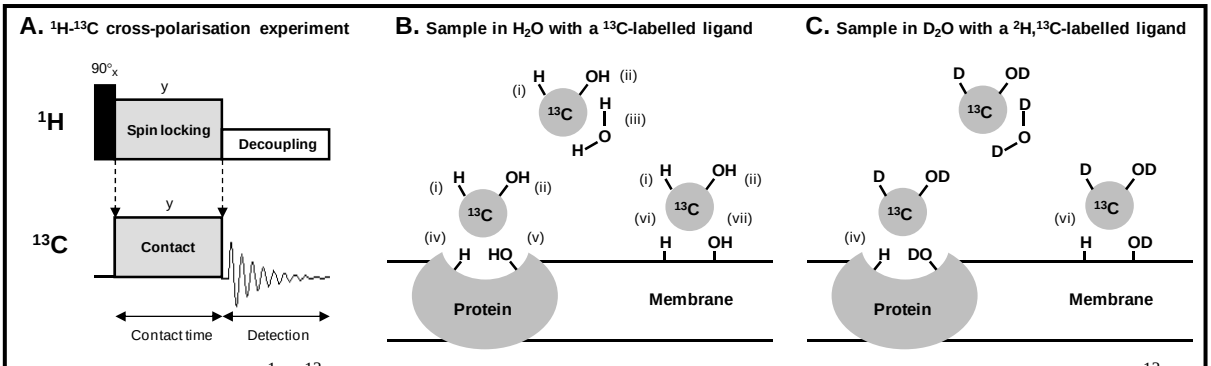


**Figure 1:** sources of $^{1}H$-$^{13}C$ CP interactions that can contribute to the signal for a weakly-binding $^{13}C$-labelled ligand in a membrane protein sample under frozen conditions using solid-state NMR (see text above for details).

the $^{1}H$-$^{13}C$ pathways that can contribute a non-specific component to the ligand signal can be eliminated with the sample in $D_2O$ and using a $^{2}H$,$^{13}C$-labelled ligand. Notably this step includes removal of the interactions associated with unbound ligand.

We have investigated this experimentally by performing $^{1}H$-$^{13}C$ CP-MAS NMR measurements at a temperature of -15 ºC on samples of *E. coli* inner membranes containing the sugar transport protein GalP with its substrate D-glucose. This work has used samples in both $H_2O$ and $D_2O$, using both D-[$^{13}C_6$]glucose and D-[$^{2}H_7$,$^{13}C_6$]glucose, and the GalP inhibitor forskolin (example spectra are shown in Figure 2). We have also performed 2D $^{13}C$-$^{13}C$ DARR and $^{1}H$-$^{13}C$ HETCOR dipolar recoupling experiments using these novel sample conditions.

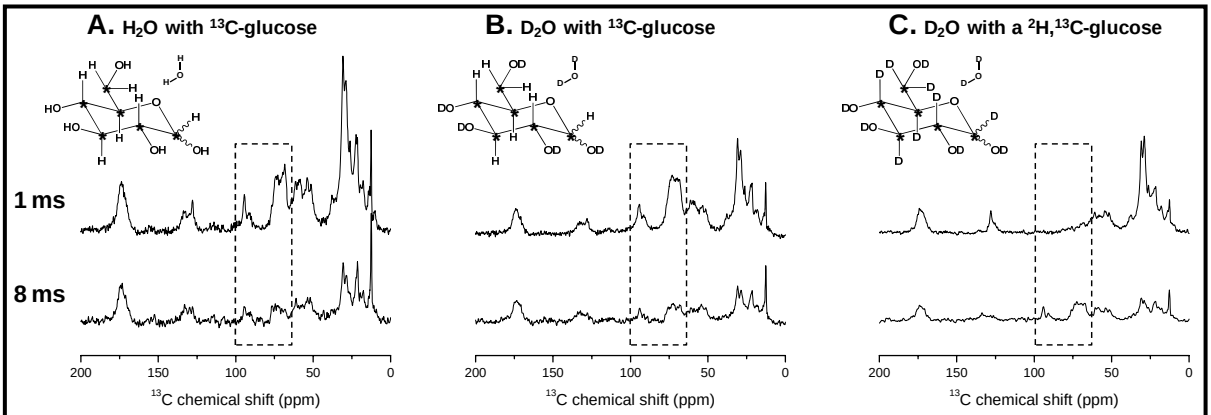


**Figure 2:** $^{13}C$ CP-MAS solid-state NMR spectra of *E. coli* inner membranes containing the sugar transport protein GalP and its substrate D-glucose at -15 ºC. **A.** is a sample in $H_2O$ with D-[$^{13}C_6$]glucose. **B.** is a sample in $D_2O$ with D-[$^{13}C_6$]glucose. **C.** is a sample in $D_2O$ with D-[$^{2}H_7$,$^{13}C_6$]glucose. Spectra were acquired using CP contact times of both 1 ms and 8 ms. The dashed boxes highlight the signals for $^{13}C$ labels in D-glucose.

## Publications

This work is being written up for publication.


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## Collaborators

This work was performed in collaboration with David Middleton at the University of Liverpool.